Assessment of Quality Changes in Frozen Sardine (*Sardina pilchardus*) by Fluorescence Detection

Santiago P. Aubourg*, Carmen G. Sotelo, and Ricardo Pérez-Martín

Instituto de Investigaciones Marinas (CSIC), C/ E. Cabello, 6, 36208-VIGO, Spain

ABSTRACT: The formation of fluorescent compounds was tested as a quality assessment during the frozen storage of sardine at –18°C (up to 24 mon) and at –10°C (up to 120 d). The fluorescence ratio between two excitation/emission maxima (393/463 and 327/415 nm) was studied in the aqueous (δF_{aq}) and organic (δF_{or}) extracts after Bligh and Dyer extraction of the white muscle. Fluorescence results were compared to common quality indices [total volatile base-nitrogen (TVB-N); conjugated dienes; thiobarbituric acid index (TBA-i); and free fatty acids (FFA)]. δF_{aq} showed good correlations with storage time ($r = 0.80$ and $r = 0.72$ at –18 and –10°C, respectively) and TBA-i ($r = 0.92$ and $r = 0.81$). Principal-component analysis grouped δF_{aq} with quality indices that are sensitive for the assessment of fish damage during frozen storage at both temperatures (TBA-i and FFA at –18°C; BVT-N, TBA-i, and FFA at –10°C). According to these results, fluorescence detection of interaction compounds in the aqueous phase can provide an accurate method to assess quality differences during frozen storage of fish.
JAOCS 75, 575–580 (1998).

KEY WORDS: Fluorescence, frozen storage, interaction compounds, lipid damages, sardine.

Canned fish and other marine species are products of great economic importance in many countries. Many of the problems encountered with poor-quality canned fish can be related to the quality of the raw material (1). Frozen storage is the most utilized method in canneries for preserving fish prior to canning. Fish species employed possess a high lipid content (2,3) and also a high proportion of polyunsaturated fatty acids (PUFA) typical of marine lipids (4). During the storage period, enzymatic and nonenzymatic lipid oxidation (5,6) can become important factors responsible for fish damage. A close relationship has been found between lipid damage and the quality of the raw material employed for canning and of the final canned product (7,8).

Many methods have been used to measure lipid oxidation in foods as a means for determining the degree of damage (9,10). However, there are some difficulties with common methods when quality has to be assessed, because oxidation products are unstable and tend to react with biological amino constituents (proteins, peptides, free amino acids, and phospholipids), leading to interaction compounds (11–14).

Analysis of the above interaction products by fluorescence detection has become a complementary method to the other, more sophisticated measurements for assessing lipid damage (15–18). Recent studies have measured the fluorescent properties of thermally treated fish at different excitation/emission maxima (19–21). Increments of lipid oxidation, time, and temperature of processing produced a fluorescence shift toward higher wavelength maxima. The fluorescence ratio between two of these maxima (393/463 and 327/415 nm) showed an interesting correlation with fish quality after cooking and during chilling (20–22).

The present work was designed to test the fluorescent compounds' detection as a quality assessment index for frozen stored sardine. Two frozen storage temperatures (−18 and −10°C) were studied. The fluorescence shift, measured as the above-mentioned ratio, was studied together with common quality estimations [total volatile base-nitrogen (TVB-N), conjugated dienes (CD), thiobarbituric acid index (TBA-i), and free fatty acids (FFA)].

MATERIALS AND METHODS

Raw material, freezing, frozen storage, and sampling. Fresh sardines (*Sardina pilchardus*) were obtained in a local market. Upon arrival in our laboratory, individual fish were frozen at −40°C and then distributed into two storage conditions: −18 and −10°C. For each storage temperature, sardines were divided into three batches, which were analyzed separately during the whole experiment. Sardines stored at −18°C were sampled after 0.5, 2, 4, 8, 12, 18, and 24 mon, and those stored at −10ºC were sampled at 3, 10, 25, 60, and 120 d. In each batch at each storage temperature, analyses were performed on the homogenized white muscle from three individual sardines.

Water and lipid contents. Water content was determined by weight difference of the homogenized muscle (1–2 g) before and after 24 h at 105°C. Lipids were extracted by the Bligh and Dyer (23) method. Quantitation was carried out according to Herbes and Allen (24).

TVB-N determination. TVB-N was measured by the Antonacopoulos (25) method with some modifications. Fish

*To whom correspondence should be addressed.
E-mail: saubourg@iim.csic.es.

muscle (10 g) was extracted with perchloric acid (6%) and made up to 50 mL. TVB-N content was determined by steam distillation of the acid extracts that were made alkaline to pH 13 with NaOH (20%), followed by titration of the distillate with 10 mM hydrochloric acid. Data were expressed as mg TVB-N/100 g muscle.

Determination of lipid damage. CD formation was measured at 233 nm (10). Results are expressed according to the following formula (15): $CD = B \times V/w$, where B is the absorbance reading at 233 nm, V denotes the volume (mL) of the sample, and w is the mass (mg) of the lipid sample.

TBA-i (mg malondialdehyde/kg sample) was determined according to Vyncke (26).

FFA content was determined by the Lowry and Tinsley (27) method, based on complex formation with cupric acetate-pyridine. Results are expressed as g FFA/100 g lipids.

Fluorescence analysis. A Perkin-Elmer LS 3B fluorescence spectrophotometer was employed. Fluorescence formation was studied at 393/463 and 327/415 nm according to previous experiences (21,22). The relative fluorescences (F_r) were calculated as: $F_r = F/F_{st}$, where F is the sample fluorescence at each excitation/emission maximum, and F_{st} is the corresponding fluorescence intensity of a quinine sulfate solution (1 μg/mL in 0.05 M H_2SO_4). The fluorescence shift (δF) was calculated as the ratio between both F_r values: $\delta F = F_{r;393/463\ nm}/F_{r;327/415\ nm}$. The δF value was studied in the aqueous (δF_{aq}) and organic (δF_{or}) phases resulted from the lipid extraction (23); the ratio between both values ($\delta F_{or}/\delta F_{aq}$) was also evaluated.

Statistical analysis. The *Statistica* package (28) was employed. Data from the different damage measurements were subjected to the analysis of variance one-way method ($P < 0.05$), correlation analysis, and factor analysis (principal components) ($P < 0.05$ and $P < 0.01$). A varimax normalized rotation was employed for factor rotation.

RESULTS

Water contents ranged between 68 and 71% in all samples. No significant differences ($P < 0.05$) were obtained as a result of the temperature of frozen storage (−18 and −10°C). Lipid contents ranged between 4 and 7% (wet basis). Small differences between samples could be explained as a result of lipid content variation in individual fish and not as a result of frozen storage.

Quality measurements. Sardines stored at –18°C produced TVB-N during the storage period at a slower rate than those stored at –10°C. A slight increase with storage time was observed at –10°C, while no clear tendency was detected at –18°C. The amount of volatile amine nitrogen has been widely employed for the estimation of fish quality during and after several processes (29,30). For frozen stored fish, volatile amines result from the breakdown of either trimethylamine oxide (for gadoid fish) or amino acids, which lead to the accumulation of dimethylamine and NH_3, respectively (31). Because nongadoid fish do not show trimethylamine oxide demethylase activity (32), the TVB-N formed during the frozen storage of sardines might be expected to come mainly from the deamination of amino acids.

Primary oxidation products (CD) showed some significant changes, although a definite pattern during storage at either frozen temperature could not be observed (Tables 1 and 2). We concluded that CD content was not suitable as a quality measurement because dienes are relatively unstable and capable of interacting with other constituents (21,22,33).

Secondary lipid oxidation products were measured by TBA-i. TBA-i determined at –18°C (Table 1) showed a significant increase after 4 mon of storage. Thereafter, progressive increases were found during the whole storage time. A similar behavior was obtained at –10°C (Table 2), where a significant increase was obtained after 10 d of storage. Ac-

TABLE 1
Quality Measurements[a] and Fluorescence Shifts[a] During Frozen Storage at −18°C[b]

ST (months)	TVB-N	CD	TBA-i	FFA	δF_{or}	δF_{aq}	$\delta F_{or}/\delta F_{aq}$
0	25.6[a]	1.2[b,c]	0.5[a]	1.1[a]	0.4[a]	0.4[a]	1.2[b]
	(0.67)	(0.12)	(0.05)	(0.14)	(0.04)	(0.02)	(0.10)
0.5	24.2[a]	1.5[c,d]	1.6[a,b]	4.2[b]	1.7[b]	0.3[a]	5.1[d]
	(0.26)	(0.14)	(0.28)	(0.63)	(0.20)	(0.02)	(0.33)
2	29.5[b]	0.9[a,b]	2.8[a,b]	3.0[b]	1.9[b]	0.3[a]	5.7[d]
	(0.52)	(0.03)	(0.61)	(0.33)	(0.14)	(0.02)	(0.64)
4	27.2[a,b]	1.2[b,c]	5.0[b,c]	4.1[b]	1.5[b]	1.2[a,b]	1.3[b]
	(1.08)	(0.01)	(0.87)	(0.20)	(0.02)	(0.10)	(0.13)
8	24.1[a]	0.8[a,b]	3.9[a,b]	4.0[b]	2.4[b]	1.0[a,b]	2.3[c]
	(0.96)	(0.07)	(0.11)	(0.16)	(0.40)	(0.07)	(0.30)
12	35.6[c]	2.2[e]	8.6[c]	7.1[c]	2.3[b]	2.4[b]	0.9[a,b]
	(0.30)	(0.37)	(0.67)	(0.37)	(0.60)	(0.33)	(0.15)
18	29.6[b]	1.9[d,e]	9.6[c]	6.4[c]	3.7[c]	5.9[c]	0.6[a,b]
	(2.63)	(0.14)	(2.10)	(0.60)	(0.15)	(0.42)	(0.04)
24	30.2[b]	0.7[a]	29.9[d]	11.8[d]	2.2[b]	12.6[d]	0.2[a]
	(0.56)	(0.03)	(2.32)	(0.63)	(0.45)	(1.28)	(0.06)

[a]Mean values of three determinations. Values in the same column followed by different letters are significantly different ($P < 0.05$). Standard errors of the means are indicated in brackets.
[b]Abbreviations: ST (storage time), TVB-N (total volatile base-nitrogen), CD (conjugated dienes), TBA-i (thiobarbituric acid index), FFA (free fatty acids), δF_{or} (fluorescence shift in the organic phase), δF_{aq} (fluorescence shift in the aqueous phase).

TABLE 2
Quality Measurements[a] and Fluorescence Shifts[a] During Frozen Storage at −10°C[b]

ST (days)	TVB-N	CD	TBA-i	FFA	δF_{or}	δF_{aq}	$\delta F_{or}/\delta F_{aq}$
0	25.6[a]	1.2[a,b,c]	0.5[a]	1.1[a]	0.4[a]	0.4[a]	1.2[a]
	(0.67)	(0.12)	(0.05)	(0.14)	(0.04)	(0.02)	(0.10)
3	28.5[c]	1.4[c]	1.9[a,b]	3.8[b]	0.7[a]	0.3[a]	2.6[a]
	(1.13)	(0.06)	(0.16)	(0.47)	(0.05)	(0.02)	(0.23)
10	22.2[a]	1.3[b,c]	2.9[b]	3.1[a,b]	2.0[b]	0.3[a]	8.1[c]
	(0.52)	(0.16)	(0.41)	(0.52)	(0.20)	(0.02)	(1.36)
25	29.3[c,d]	1.1[a,b]	5.4[c]	6.9[c]	1.8[b]	0.4[a]	5.2[b]
	(0.45)	(0.02)	(0.41)	(0.40)	(0.08)	(0.05)	(0.65)
60	28.4[c]	1.0[a]	5.2[c]	7.8[c]	3.1[c]	0.5[b]	5.7[b]
	(0.93)	(0.07)	(0.20)	(1.74)	(0.59)	(0.07)	(0.93)
120	31.1[d]	1.4[c]	8.7[d]	8.5[c]	1.8[b]	2.2[c]	0.8[a]
	(0.28)	(0.09)	(1.07)	(0.07)	(0.14)	(0.05)	(0.08)

[a]Mean values of three determinations. Values in the same column followed by different letters are significantly different ($P < 0.05$). Standard errors of the means are indicated in brackets.
[b]See Table 1 for abbreviations.

cording to previous experiences (34,35), TBA-i is an accurate way of assessing quality changes during frozen storage time.

Lipid hydrolysis was measured by FFA. A progressive increase of FFA content was observed throughout storage at both frozen temperatures (Tables 1 and 2). At −18°C, increases were detected after 0.5, 12, and 24 mon of storage. At −10°C, differences were observed after 3 and 25 d of storage. According to previous experiences (34,36), FFA formation as a result of lipid hydrolysis (triglyceride and phospholipid classes) has provided a suitable means for assessment of fish damage during frozen storage.

Fluorescence measurements. The fluorescence shift, measured in the organic phase that resulted from the Bligh and Dyer (23) extraction (δF_{or}) of the different frozen samples, was studied. Both storage temperatures (Tables 1 and 2) showed a progressive increase of the δF_{or} value with time, except at the end of storage where a decrease was detected (24 mon at –18°C and 120 d at –10°C). Significant increases were obtained after 0.5 and 18 mon (at –18°C) and after 10 and 60 d (at –10°C). However, measurement of fluorescence in the aqueous phase, resulting from Bligh and Dyer (23) extraction (δF_{aq} value), showed a progressive increase along the whole storage time at both temperatures (Tables 1 and 2). At −18°C, significant increases were detected after 12, 18, and 24 mon, while at −10°C, increases were observed after 60 and 120 d.

To study the relative formation of fluorescent compounds that are soluble in organic solvents and water, the $\delta F_{or}/\delta F_{aq}$ ratio was evaluated. A similar behavior was obtained at both frozen temperatures (Tables 1 and 2). An increase in the $\delta F_{or}/\delta F_{aq}$ ratio was observed during the first periods of storage, but it was followed by a sharp decrease after 12 mon (at –18°C) and after 25 d (at –10°C).

A similar behavior was observed by studying the $\delta F_{or}/\delta F_{aq}$ ratio during sardine storage at 0 and 10°C (22). In the above work, during the first steps of storage, fluorescent compounds responsible for the δF value were mostly lipid-soluble; however, as lipid damage increased, these kinds of compounds became progressively more soluble in the aqueous phase.

Fluorescent compound studies based on a single excitation/emission maximum have been mostly carried out on organic extracts (lipids) and have shown high correlations with sensory measurements and storage times (16,17). However, experimental evidence has demonstrated that fluorescent substances formed from oxidized membrane lipids remain attached to the amino constituents and result in compounds that are quite insoluble in organic solvents (37–39). According to our results based on fluorescence ratios, analysis of the aqueous phase can provide a more accurate assessment of the fluorescent properties of the interaction compounds formed as a result of fish damage.

Correlation and multivariate analyses. The different damage indices were tested for correlations with storage time (ST) and also with each other (Tables 3 and 4). Almost every index correlates well with ST at both temperatures, except for the CD, which did not vary with ST. The indices behave similarly at both storage temperatures. Two indices (TBA-i and FFA) correlate best with ST at both temperatures and, according to previous experiences, could be used as an indication of quality deterioration of frozen sardine (34–36).

Concerning the fluorescence measurements, both indices (δF_{or} and δF_{aq}) reflected good correlations with ST at both temperatures and were strongly correlated with those lipid damage indices that also show variation with storage time

TABLE 3
Correlation Matrix for Different Parameters (storage time and quality estimations) Measured During Sardine Storage at −18°C[a]

	TVB-N	CD	TBA-i	FFA	δF_{or}	δF_{aq}
ST	0.52[b]	0.09	0.79[c]	0.82[c]	0.65[b]	0.80[c]
TVB-N		0.43[b]	0.36	0.46[b]	0.35	0.34
CD			–0.22	–0.10	0.23	–0.15
TBA-i				0.87[c]	0.35	0.92[c]
FFA					0.50[b]	0.83[c]
δF_{or}						0.36

[a]See Table 1 for abbreviations.
[b]Significance: $P < 0.05$.
[c]Significance: $P < 0.01$.

TABLE 4
Correlation Matrix for Different Parameters (storage time and quality estimations) Measured During Sardine Storage at –10°C[a])

	TVB-N	CD	TBA-i	FFA	δF_{or}	δF_{aq}
ST	0.55[b]	–0.07	0.94[c]	0.88[c]	0.72[b]	0.72[b]
TVB-N		0.07	0.61[b]	0.71[b]	0.14	0.60[b]
CD			0.14	–0.03	–0.24	0.27
TBA-i				0.84[c]	0.56[b]	0.81[c]
FFA					0.73[b]	0.59[b]
δF_{or}						0.17

[a]See Table 1 for abbreviations.
[b]Significance: $P < 0.05$.
[c]Significance: $P < 0.01$.

(FFA and TBA-i). Correlation data are better for δF_{aq} than for δF_{or}, especially after –18°C storage.

Principal-component analysis showed that 80.0 and 80.9% of the variability observed at –18 and –10°C, respectively, could be explained by two factors. These factor loadings are graphically displayed in Figures 1 and 2. Figure 1 (–18°C) shows that ST, FFA, TBA-i, and δF_{aq} are grouped together at high loading in the Factor 1 axis, whereas their loading values for Factor 2 are low; meanwhile CD, TVB-N, and δF_{or} clearly have higher loadings in Factor 2. At –10°C (Fig. 2), similar results can be observed, with the exception that the TVB-N index has a high loading in the Factor 1 axis and is grouped together with ST, FFA, TBA-i, and δF_{aq}.

DISCUSSION

In previous experiences related to chilling, cooking, and canning, lipid deterioration compounds (peroxides and carbonyls) have caused the formation of interaction compounds with fluorescent properties (20–22). Accelerated by the temperature, fluorescent compounds formed in the first stages of interaction led to the formation of other fluorescent compounds, which showed excitation/emission maxima at higher wavelengths than their precursors. The fluorescence ratio between two of these maxima (393/463 and 327/415 nm) provided an interesting method for quality assessment.

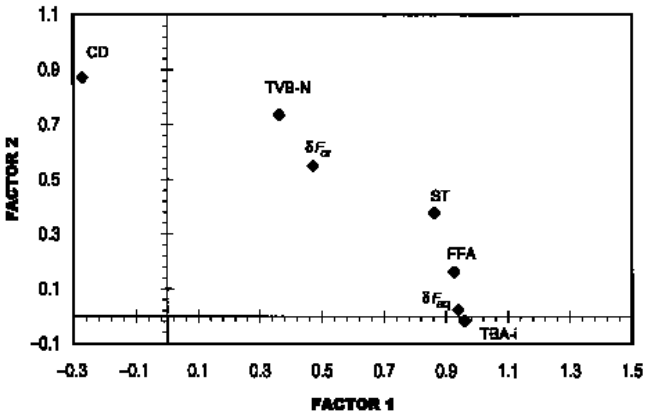

FIG. 1. Principal-component analysis for different parameters (storage time and quality indices) measured at –18°C storage. Abbreviations: ST (storage time), TVB-N (total volatile base-nitrogen), CD (conjugated dienes), TBA-i (thiobarbituric acid index), FFA (free fatty acids), δF_{or} (fluorescence shift in the organic phase), δF_{aq} (fluorescence shift in the aqueous phase).

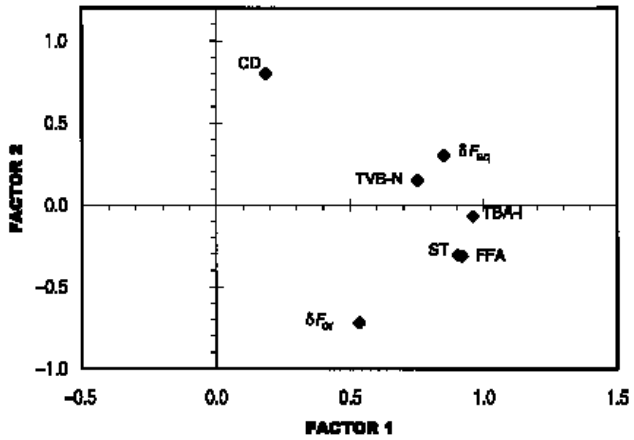

FIG. 2. Principal component analysis for different parameters (storage time and quality indices) measured at –10°C storage. See Figure 1 for abbreviations.

The aim of the present work was to study fluorescence formation during frozen storage and to test the fluorescence ratio as a way of assessing fish quality changes. Previous research on frozen storage (40,41) had detected some fluorescence that could be related to the quality of product.

In the present experiment, detection by fluorescence of interaction compounds was accomplished in both the aqueous and organic phases that resulted from the lipid extraction. As for chilling, fluorescence analysis of the aqueous phase (δF_{aq}) provided better results than that of the organic phase (δF_{or}). δF_{aq} showed a good correlation with storage time and was grouped together with indices that reflect fish damage at –18°C (TBA-i and FFA) and at –10°C (TBA-i, FFA, and TVB-N).

The satisfactory correlation of fluorescence measurements with secondary lipid oxidation (TBA-i) is in accord with the general theory about fluorescent compound formation as a result of interaction between carbonyl compounds (electrophilic molecules), formed during lipid oxidation, and amine compounds (nucleophilic molecules) that are present in fish muscle (13,14,42).

A good correlation between lipid hydrolysis (FFA) and lipid oxidation (TBA-i) has also been obtained. Some previous experiences have proved the influence of lipid hydrolysis on lipid oxidation (43,44). According to the correlations obtained, a positive influence of the FFA content on fluorescent compound formation can be inferred.

Results obtained in this work reinforce the role of fluorescence detection of interaction compounds as a tool for quality assessment, especially when processing food with a high content of unsaturated lipids and amino compounds. This is the situation in marine species that are known to contain high amounts of PUFA (4) and free amino nitrogen compounds (45).

ACKNOWLEDGMENTS

The authors thank Oscar Bellón and Marcos Trigo for technical assistance and the Xunta de Galicia for financial support (Project XUGA 402 01B93).

REFERENCES

1. Cheftel, J., and H. Cheftel, *Introduction to the Biochemistry and Technology of Foods*, Editorial Acribia, Zaragoza, 1976.
2. Hale, M., and T. Brown, Fatty Acids and Lipid Classes of Three Underutilized Species and Change Due to Canning, *Marine Fish. Rev. 45*:4–6 (1983).
3. Aubourg, S., C. Sotelo, and J. Gallardo, Changes in Flesh Lipids and Fill Oils of Albacore (*Thunnus alalunga*) During Canning and Storage, *J. Agric. Food Chem. 38*:809–812 (1990).
4. Ackman, R., Fatty Acids, in *Marine Biogenic Lipids, Fats and Oils,* edited by R. Ackman, CRC Press, Boca Raton, FL, 1989, Vol. 1, pp. 103–137.
5. Hsieh, R., and J. Kinsella, Oxidation of Polyunsaturated Fatty Acids: Mechanisms, Products and Inhibition with Emphasis on Fish, *Adv. Food Res. Nutr. Res. 33*:233–341 (1989).
6. German, B., H. Zhang, and R. Berger, Role of Lipoxygenases in Lipid Oxidation in Foods, in *Lipid Oxidation in Food*, edited by A. St. Angelo, American Chemical Society, Washington, D.C., 1992, pp. 74–92.
7. Pearson, A., J. Love, and F. Shorland, Warmed-Over Flavor in Meat, Poultry and Fish, *Adv. Food Res. 23*:2–61 (1977).
8. Pigott, G., and B. Tucker, Science Opens New Horizons for Marine Lipids in Human Nutrition, *Food Rev. Intern. 3*:105–138 (1987).
9. Melton, S., Methodology for Following Lipid Oxidation in Muscle Foods, *Food Technol. 37*:105–111 (1983).
10. Kim, R., and F. Labella, Comparison of Analytical Methods for Monitoring Autoxidation Profiles of Authentic Lipids, *J. Lipid Res. 28*:1110–1117 (1987).
11. Pokorny, J., Interactions of Oxidized Lipids with Proteins, *Riv. Ital. Sostanze Grasse 54*:389–393 (1977).
12. Gardner, H., Lipid Hydroperoxide Reactivity with Proteins and Amino Acids: A Review, *J. Agric. Food Chem. 27*:220–229 (1979).
13. Leake, L., and M. Karel, Nature of Fluorescent Compounds Generated by Exposure of Protein to Oxidizing Lipids, *J. Food Biochem. 9*:117–136 (1985).
14. Kikugawa, K., and M. Beppu, Involvement of Lipid Oxidation Products in the Formation of Fluorescent and Cross-Linked Proteins, *Chem. Phys. Lipids 44*:277–297 (1987).
15. Smith, G., M. Hole, and S. Hanson, Assessment of Lipid Oxidation in Indonesian Salted-Dried Marine Catfish (*Arius thalassinus*), *J. Sci. Food Agric. 51*:193–205 (1990).
16. Maruf, F., D. Ledward, R. Neale, and R. Poulter, Chemical and Nutritional Quality of Indonesian Dried-Salted Mackerel (*Rastrelliger kanagurta*), *Int. J. Food Sci. Technol. 25*:66–77 (1990).
17. Lubis, Z., and K. Buckle, Rancidity and Lipid Oxidation of Dried-Salted Sardines, *Ibid. 25*:295–303 (1990).
18. Miyagawa, K., K. Hirai, and R. Takeoze, Tocopherol and Fluorescence Levels in Deep-Frying Oil and Their Measurement for Oil Assessment, *J. Am. Oil Chem. Soc. 68*:163–166 (1991).
19. Aubourg, S., R. Pérez-Martín, I. Medina, and J. Gallardo, Fluorescence Formation by Interaction of Albacore (*Thunnus alalunga*) Muscle with Acetaldehyde in a Model System, *J. Agric. Food Chem. 40*:1805–1808 (1992).
20. Aubourg, S., R. Pérez-Martín, I. Medina, and J. Gallardo, Fluorescence Formation During Albacore (*Thunnus alalunga*) Thermal Processing, *Z. Lebensm. Unters. Forsch. 195*:332–335 (1992).
21. Aubourg, S., I. Medina, and R. Pérez-Martín, A Comparison Between Conventional and Fluorescence Detection Methods of Cooking-Induced Damage to Tuna Fish Lipids, *Z. Lebensm. Unters. Forsch. 200*:252–255 (1995).
22. Aubourg, S., C. Sotelo, and J. Gallardo, Quality Assessment of Sardines During Storage by Measurement of Fluorescent Compounds, *J. Food Sci. 62*:295–299 (1997).
23. Bligh, E., and W. Dyer, A Rapid Method of Total Extraction and Purification, *Can. J. Biochem. Physiol. 37*:911–917 (1959).
24. Herbes, S., and C. Allen, Lipid Quantification of Freshwater Invertebrates: Method Modification for Micro Quantification, *Can. J. Fish. Aquat. Sci. 40*:1315–1317 (1983).
25. Antonacopoulos, N., Verbesserte Apparatus zur Quantitativer Destillation Wasserdampfflühtiger Stoffe*, Z. Lebensm. Unters. Forsch. 113*:113–160 (1960).
26. Vyncke, W., Direct Determination of the Thiobarbituric Acid Value in Trichloracetic Acid Extracts of Fish as a Measure of Oxidative Rancidity, *Fette Seifen Anstrichm. 72*:1084–1087 (1970).
27. Lowry, R., and I. Tinsley, Rapid Colorimetric Determination of Free Fatty Acids, *J. Am. Oil Chem. Soc. 53*:470–472 (1976).
28. *Statistica for Macintosh*, Statsoft, Tulsa, OK, 1994.
29. Gallardo, J., S. Aubourg, and R. Pérez-Martín, Lipid Classes and Their Fatty Acids at Different Loci of Albacore (*Thunnus alalunga*): Effects of Precooking, *J. Agric. Food Chem. 37*: 1060–1064 (1990).
30. Bennour, M., A. El Marrakchi, N. Bouchriti, A. Hamama, and M. El Ouadaa, Chemical and Microbiological Assessments of Mackerel (*Scomber scombrus*) Stored in Ice, *J. Food Protect. 54*:784–792 (1991).
31. Sotelo, C., C. Piñeiro, and R. Pérez-Martín, Denaturation of Fish Proteins During Frozen Storage: Role of Formaldehyde, *Z. Lebensm. Unters. Forsch. 200*:1–2 (1995).
32. Rehbein, H., Relevance of Trimethylamine Oxide Demethylase Activity and Haemoglobin Content to Formaldehyde Production and Texture Deterioration in Frozen Stored Minced Fish Muscle*, J. Sci. Food Agric. 43*:261–276 (1988).
33. Shimasaki, H., O. Privett, and I. Hara, Studies of the Fluorescent Products of Lipid Oxidation in Aqueous Emulsion with Glycine and on the Surface of Silica Gel, *J. Am. Oil Chem. Soc. 54*:119–123 (1977).
34. Quaranta, H., and S. Pérez, Chemical Methods for Measuring Changes in Freeze Stored Fish: A Review, *Food Chemistry 11*: 79–85 (1983).
35. Orlick, B., J. Oehlenschläger and W. Schreiber, Changes in Lipids and Nitrogenous Compounds in Cod (*Gadus morhua*) and Saithe (*Pollachius virens*) During Frozen Storage, *Arch. Fish. Wiss. 41*:89–99 (1991).
36. de Koning, A., and T. Mol, Quantitative Quality Tests for Frozen Fish: Soluble Protein and Free Fatty Acid Content as Quality Criteria for Hake (*Merluccius merluccius*) Stored at –18°C, *J. Sci. Food Agric. 54*:449–458 (1991).
37. Shimasaki, H., N. Ueta, H. Mowri, and K. Inove, Formation of Age Pigment-Like Fluorescent Substances During Peroxidation of Lipids in Model Membranes, *Biochim. Biophys. Acta 792*: 123–129 (1984).
38. Iio, T., and K. Yoden, Fluorescence Formation from Hydroperoxide of Phosphatidylcholine with Amino Compound, *Lipids 23*:65–67 (1988).
39. Hasegawa, K., Y. Endo, and K. Fujimoto, Oxidative Deterioration in Dried Fish Model Systems Assessed by Solid Sample Fluorescence Spectrometry, *J. Food Sci. 57*:1123–1126 (1992).
40. Davies, H., Fluorescence of Fish Muscle: Description and Measurement of Changes Occurring During Frozen Storage, *J. Sci. Food Agric. 33*:1135–1142 (1982).
41. Davies, H., and P. Reece, Fluorescence of Fish Muscle: Causes

of Change Occurring During Frozen Storage, *Ibid. 33*: 1143–1151 (1982).
42. Tappel, A., Measurement of and Protection from *in vivo* Lipid Peroxidation, in *Free Radicals in Biology*, edited by R. Ackman, Academic Press, New York, 1980, Vol. 4, pp. 1–47.
43. Miyashita, K., and T. Takagi, Study on the Oxidative Rate and Prooxidant Activity of Free Fatty Acids, *J. Am. Oil Chem. Soc. 63*:1380–1384 (1986).
44. Han, T., and J. Liston, Correlation Between Lipid Peroxidation and Phospholipid Hydrolysis in Frozen Fish Muscle, *J. Food Sci. 53*:1917–1918 (1988).
45. Navarro, P., Valor Nutritivo del Pescado: I. Pescado Fresco, *Rev. Agroquím Tecnol. Aliment. 31*:330–342 (1991).

[Received July 28, 1997; accepted December 1, 1997]